

Summer and Autumn Growth of Rhizomatous Birdsfoot Trefoil

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ABSTRACT

A new population of birdsfoot trefoil (*Lotus corniculatus* L.) with rhizomes (RBFT) has been developed for greater persistence. No study has compared RBFT with a standard, non-rhizomatous cultivar of birdsfoot trefoil (BFT). Objectives were to: (i) compare RBFT with BFT for differences in shoot and root mass and total nonstructural carbohydrate (TNC) concentration of taproots in both clipped and non-clipped situations and (ii) describe rhizome production of clipped and non-clipped RBFT. 'Norcen' BFT and RBFT were grown in field plots near Columbia, MO, in 1994 and 1995. The study had four treatments: non-clipped RBFT, clipped RBFT, non-clipped BFT, and clipped BFT. Each treatment was replicated four times in a randomized complete block. Plots were sampled biweekly for shoot and root mass and taproot TNC from early-July until the first killing frost in October. In addition, length, mass, TNC, and number of rhizomes per plant were recorded for RBFT. From mid-September until the final sampling, the shoot mass for RBFT was about half that of BFT; however, RBFT's shoot mass was less affected by clipping than was BFT's. Taproot TNC was 20 to 40 g kg⁻¹ greater for RBFT than BFT throughout both growing seasons. Nearly all RBFT plants exhibited rhizomes by mid-October both years. Clipping RBFT plants did not affect rhizome growth. Rhizome TNC concentration increased steadily during autumn, with a final concentration of approximately 220 g kg⁻¹. The failure of clipping to decrease rhizome production, combined with higher levels of below-ground TNC, may give RBFT the ability to withstand frequent defoliation.

BIRDSFOOT TREFOIL is a perennial legume used for hay and pasture throughout the world. It tolerates acid, infertile, poorly drained soils, and provides nutritious forage for livestock (Beuselinck and Grant, 1995). Unfortunately, birdsfoot trefoil persists poorly throughout the central and southern USA. Several root- and crown-rotting pathogens attack birdsfoot trefoil, causing as much as 90% stand mortality in less than 3 yr after establishment (Beuselinck et al., 1984). Plant breeders and agronomists have tried to improve stand persistence by developing cultivars resistant to root- and crown-rot or encouraging natural reseeding, but these efforts have had only moderate success (Beuselinck et al., 1984).

A new population of birdsfoot trefoil with rhizomes offers an additional strategy to improve stand persistence (Beuselinck, 1994; Li and Beuselinck, 1996). Rhizome production could mitigate the damage done by disease because as older plants die from root- and crown-rot, "daughter" plants, naturally propagated from rhizomes and independent of their parent, would continue to grow. Thus, even a disease-ridden stand

might persist indefinitely if new plants were produced as fast as the old plants succumbed to disease.

In other cool-season perennial legumes, the presence of rhizomes has been shown to increase stand persistence and tolerance to defoliation. *Lotus uliginosis* Schk., another species that has rhizomes, has enhanced stand persistence compared with birdsfoot trefoil (Beuselinck and Grant, 1995). Sheath (1981) reported that *L. uliginosis* rhizomes and associated fibrous roots developed rapidly during the autumn and fragmented over the winter and spring, resulting in the formation of new plants. Spring shoot growth of *L. uliginosis* is initiated mainly from the nodes on rhizomes formed the previous autumn (Wedderburn and Gwynne, 1981).

Little is known about the growth of rhizomatous birdsfoot trefoil and how its growth is altered by typical management practices like clipping or grazing. A more thorough understanding of shoot, root, and rhizome production during the late summer and fall is necessary for agronomists making recommendations about fall clipping and grazing management.

Our objectives were to: (i) compare RBFT with BFT for differences in shoot and root mass and TNC concentration of taproots in both clipped and non-clipped situations and (ii) describe rhizome production of clipped and non-clipped RBFT.

MATERIALS AND METHODS

Site Characteristics

This study was conducted in 1994 and 1995 at the University of Missouri Agronomy Research Center near Columbia, MO (38°53' N Lat.; 92°12' W Long.). The soil was a Mexico silt loam (fine, montmorillinitic, mesic, Udollic Ochraqualf) with a soil pH of 6.4. Soil P, K, Ca, and Mg levels were rated medium or higher according to University of Missouri Extension guidelines for the establishment of birdsfoot trefoil-grass pasture. No fertilizer was applied either year.

Experimental Design and Culture

Two populations of birdsfoot trefoil were compared in the study; a rhizomatous population designated, RBFT and a non-rhizomatous population designated, BFT. The rhizomatous population was chosen from 10 rhizomatous F1 hybrid plants generated by crossing rhizomatous accessions G 31276 and G 31317 with the non-rhizomatous commercial cultivar Norcen (BFT). Five- to 8-cm cuttings of fresh shoots from each population were rooted in vermiculite. Rooted cuttings were dipped in a slurry of commercial inoculum (Urbana Laboratories, St. Joseph, MO)¹ for *Lotus* species, and planted into 15.0-cm (1994) and 12.5-cm (1995) diam plastic pots containing a com-

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¹ Mention of trade name or proprietary product does not constitute endorsement by the University of Missouri or the USDA over the products of other manufacturers that may also be suitable.

Abbreviations: BFT, non-rhizomatous birdsfoot trefoil; RBFT, rhizomatous birdsfoot; TNC, total nonstructural carbohydrate.

mercial potting soil mix and grown in the greenhouse. Cuttings were used in this study because there was a limited supply of RBFT seed available and preliminary studies had shown that shoots and roots of RBFT and BFT plants propagated from cuttings were similar to those grown from seed (McGraw and Beuselinck, 1993, unpublished data). Insect pests were controlled in the greenhouse with a systemic drench containing 10 mL L⁻¹ imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylidiniamine]. In addition, 500 mL of Peters Professional Peat-Lite (20-10-20) (Scotts Company, Columbus, OH) mixed at a concentration of 450 mg kg⁻¹, was added twice to each pot while in the greenhouse. Excess shoot growth was removed by clipping plants to a 4-cm stubble height during the first week of April and May each year.

Both populations were transplanted on 20, 23, and 24 May in 1994 and on 23 May in 1995. A new set of plants was transplanted each year to ensure that plants were the same physiological age at comparable samplings between years. Prior to transplanting, roots of each plant were washed free of soil and trimmed to a length of 10 cm. Shoots were trimmed to a height of 6 to 8 cm at the same time. A tree-planting bar was used to place plants directly into untilled soil on a 1- by 1-m spacing. Plants were arranged in four 10- by 20-m replicates each containing 100 plants of both types.

There were four treatments in this experiment: (i) non-clipped RBFT, (ii) clipped RBFT, (iii) non-clipped BFT, and (iv) clipped BFT. The shoots from the plants in the non-clipped treatments were allowed to grow undisturbed from transplanting until sampling ended in late fall. The plants in the clipped treatments were cut to an 8-cm stubble height on 6 July and 23 August in 1994 and 5 July and 18 August in 1995.

Preexisting vegetation at the field site was controlled by a preplant application of paraquat [1:1-dimethyl-4-4'-bipyridinium dichloride]. Twenty-four days after transplanting the birdsfoot trefoil plants were covered and the plots sprayed with 24 g a.i. L⁻¹ glyphosate [*N*-(phosphonomethyl) glycine]. Further weed control was done by hand.

Sampling Procedure

Starting in early-July and ending with the first killing frost (25 October, 1994 and 21 October, 1995), samples were collected every 2 wk resulting in nine sampling dates. A randomly chosen row of five plants was dug from each treatment in each replicate for a total of 80 plants per sampling date. Plants were washed, placed in plastic bags, and stored in ice-filled chests. In the laboratory, plants were separated into shoot, root (including crown), and rhizome components. For RBFT plants, rhizomes longer than 1 cm were counted, cut, and measured at each sampling. Shoot, root, and rhizome components were then cut into 2- to 4-cm lengths and stored at -55°C. Frozen samples were freeze-dried, weighed, and then stored in airtight plastic bags at room temperature. Except for rhizomes, freeze-dried samples were ground in a cyclone mill (UDY Corp., Ft. Collins, CO) to pass a 1-mm screen. Freeze-dried rhizomes were cut into 1-cm pieces and ground with a pestle and mortar.

Taproot and Rhizome Carbohydrate Determinations

Total nonstructural carbohydrates in taproots and rhizomes were determined by near infrared reflectance spectroscopy (NIRS) using a Pacific Scientific 6250 scanning monochromator (NIRSystems, Silver Spring, MD) with software developed by Infrasoft International (Port Matilda, PA). The spectrophotometer was calibrated for TNC by regressing chemically derived data against spectral data by modified partial least

squares regression (Shenk and Westerhaus, 1991). One hundred seventy-one samples were used to develop prediction equations. The optimum equation had a coefficient of determination of 0.98 and a 1-variance ratio of 0.95. The regression mean was 118 g kg⁻¹ and standard errors of calibration and cross validation were 1.13 and 1.38 g kg⁻¹, respectively.

Total nonstructural carbohydrate was determined for the 171 calibration samples by Smith's (1981) procedures with the following modifications: 500 units of amyloglucosidase were used as the digesting enzyme, buffer solution pH was adjusted to 4.5, and incubation temperature was adjusted to 38°C. All TNC analyses were conducted in duplicate. Duplicate samples that differed more than 10 g kg⁻¹ were reanalyzed before being used to develop the NIRS calibration equation.

Statistical Design and Analysis

The experimental design was a randomized complete block with four replications. Analysis of variance was conducted on treatments, sampling dates, years, and interactions by the analysis outlined by Steel and Torrie (1980). The degrees of freedom and the error terms used to test each effect are listed in Table 1. Separation of means was conducted by Fishers protected LSD ($P = 0.05$) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Shoot Mass

During 1994 and 1995, plants grew slowly from early July until mid-August regardless of the treatment, although the shoot mass for RBFT was often less than BFT (Fig. 1). Lack of shoot growth from birdsfoot trefoil during the summer has been previously reported (Smith, 1962; Nelson and Smith, 1968; Greub and Wedin, 1971; Peterson et al., 1992).

Beginning in late August of both years, shoots began to grow faster (Fig. 1). For instance, in 1994, non-clipped RBFT more than doubled its shoot mass between 31 August and 26 October, going from 31 to 79 g plant⁻¹. By comparison, non-clipped BFT's shoot mass was

Table 1. Analysis of variance showing degrees of freedom and error terms used to test main effects and interactions for shoot mass, root mass, taproot TNC concentration, rhizome expression, rhizomes per plant, combined length of rhizomes, rhizome dry mass and rhizome TNC concentration.

| Source | df† | df‡ |
|--|-----|-----|
| Replication ($r - 1$) | 3 | 3 |
| Treatment ($t - 1$) | 3 | 1 |
| Orthogonal Contrasts | | |
| Norcen vs. RBFT | 1 | |
| Non-clipped vs. Clipped | 1 | 1 |
| (Norcen and RBFT) vs. (Non-clipped and clipped) | 1 | |
| Error a ($(r - 1)(t - 1)$) | 9 | 3 |
| Sampling date ($s - 1$) | 8 | 3 |
| Sampling date \times Treatment ($(s - 1)(t - 1)$) | 24 | 3 |
| Sampling date \times Replication ($(s - 1)(r - 1)$) | 24 | 9 |
| Error b ($(s - 1)(t - 1)(r - 1)$) | 72 | 9 |
| Year ($y - 1$) | 1 | 1 |
| Year \times Treatment ($(y - 1)(t - 1)$) | 3 | 1 |
| Error c ($(r - 1)t(y - 1)$) | 12 | 6 |
| Year \times Sampling date ($(y - 1)(s - 1)$) | 8 | 3 |
| Year \times Treatment \times Sampling date ($(y - 1)(t - 1)(s - 1)$) | 24 | 3 |
| Error d ($(r - 1)t(y - 1)(s - 1)$) | 96 | 18 |
| Grand Total (rtys - 1) | 287 | 64 |

† Degrees of freedom for shoot mass, root mass, and taproot TNC.

‡ Degrees of freedom for rhizomes per plant, combined length of rhizomes, rhizome dry mass, and rhizome TNC concentration.

about two times greater than RBFT's, weighing 58 g plant⁻¹ on 31 August and 164 g plant⁻¹ on 26 October. Results for non-clipped plants followed a similar trend the next year, although the final shoot mass was 20 to 30% less in 1995 than in 1994.

In both years, RBFT and BFT shoot mass was reduced by clipping in mid-August ($P < 0.05$). At the final sampling in 1994, clipped RBFT's shoot mass was 22% less than non-clipped RBFT's. The reduction in BFT was even larger ($P < 0.05$) than that for RBFT, as clipped BFT had 42% less shoot mass than non-clipped BFT. Although the differences between clipped and non-clipped plants were larger in 1995, the same general trend occurred. At the final sampling, clipped RBFT had a 39% reduction in shoot mass when compared with non-clipped RBFT, which was significantly ($P < 0.05$) less than the 48% reduction for clipped BFT when compared with non-clipped BFT. The smaller effect of clipping on the shoot mass of RBFT compared with BFT implies that grazing or clipping management may be less critical for the growth of RBFT. This sug-

gests that RBFT would be better suited to situations where continuous grazing or frequent clipping is practiced.

Root Mass

Root mass was nearly constant from early July through the end of August (Fig. 2). Root mass was not significantly different between the four treatments during July and August of either year, with all plants having less than 5.5 g of root mass per plant.

In September and early October 1994, non-clipped BFT had 41 to 53% more ($P < 0.05$) root mass than any of the other treatments however by the final sampling on 26 October, no other treatment was significantly different than non-clipped BFT (avg. of 31.5 g plant⁻¹). In 1995, both non-clipped RBFT and non-clipped BFT had more root mass than their clipped counterparts did on 12 and 26 September and 10 October, but by 24 October, all four treatments were again equal (avg. of 16.8 g plant⁻¹).

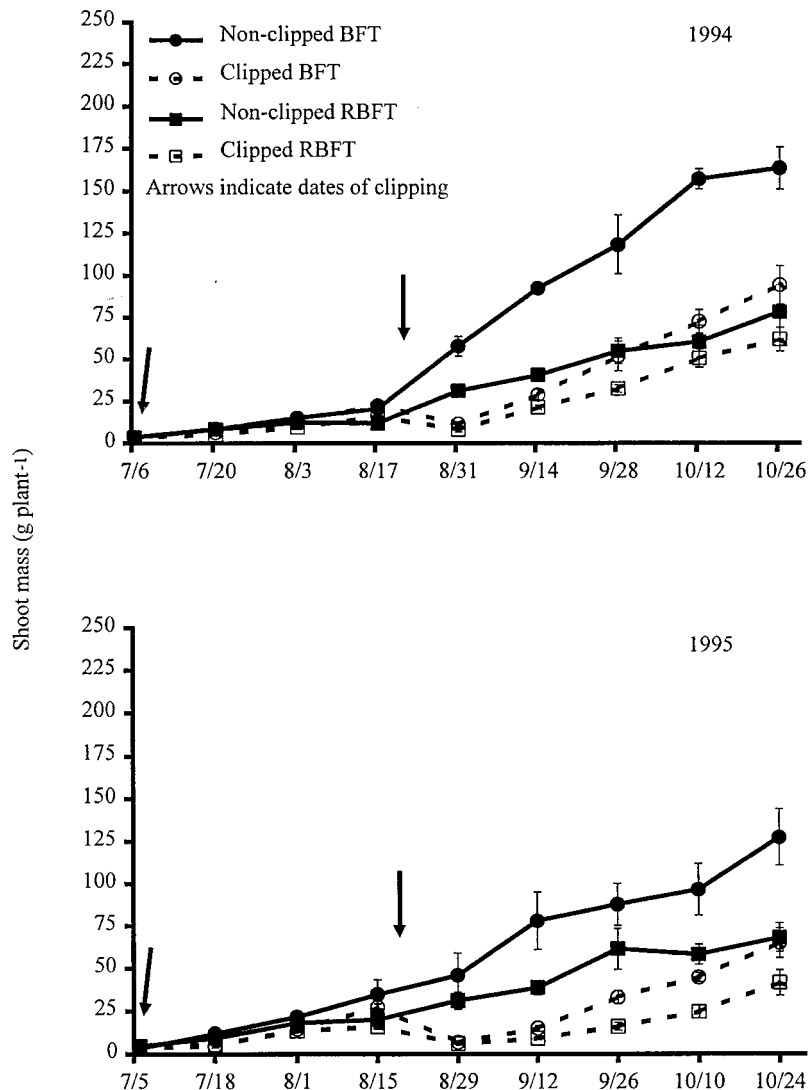


Fig. 1. Shoot mass of non-clipped and clipped RBFT and BFT plants grown at the Agronomy Research Center, near Columbia, MO. Bars show standard errors. Arrows indicate dates of clipping.

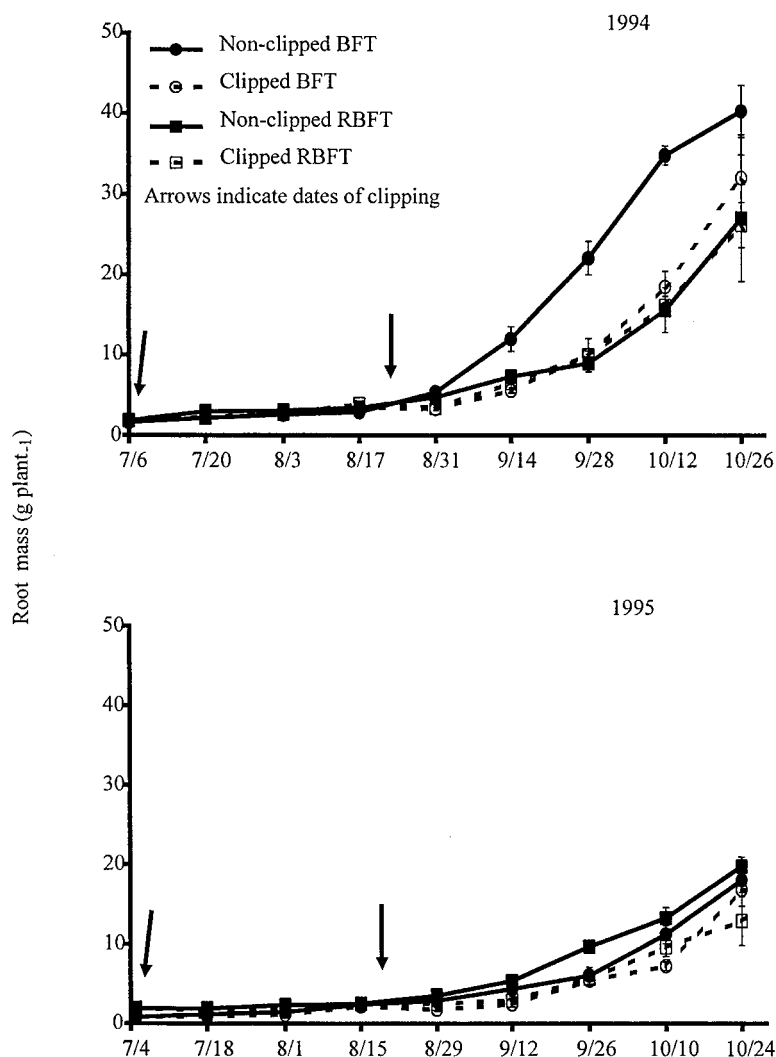


Fig. 2. Root mass of non-clipped and clipped RBFT and BFT plants grown at the Agronomy Research Center, near Columbia, MO. Bars show standard errors. Arrows indicate dates of clipping.

The final root mass for all treatments was greater ($P < 0.05$) in 1994 than in 1995. From mid-August through mid-September, the average air temperature for our site was 3°C warmer in 1995 than in 1994 (Fig. 3). In addition, precipitation during this period was 15 mm in 1995 compared with 135 mm in 1994. The warmer and drier conditions in 1995 were apparently less conducive for root growth, possibly explaining the lower root mass in 1995 compared with 1994.

Taproot TNC Concentration

Both RBFT and BFT showed little change in taproot TNC concentration from early-July through August of either year (Fig. 4). Although the taproot TNC concentration for all of the treatments was low during this time, the TNC concentration for RBFT was consistently 20 to 79 g kg⁻¹ higher ($P < 0.05$) than for BFT.

Starting in mid-September of 1994, taproot TNC concentration in all treatments began to increase and continued to increase at each subsequent sampling. In 1994, non-clipped RBFT went from 117 g kg⁻¹ on 14 Septem-

ber to over 220 g kg⁻¹ by 26 October. Non-clipped BFT increased at a similar rate (avg. of 2.69 g kg⁻¹ d⁻¹) but had 20 to 40 g kg⁻¹ less TNC. In 1994, clipping had no effect ($P > 0.05$) on taproot TNC concentration for either RBFT or BFT at any sampling date.

In 1995, taproot TNC concentration showed a trend that was similar to that for 1994, but there were some differences between years. The data for 1994 were similar to those for 1995 in that all of the treatments began to increase in taproot TNC concentration beginning in September. In 1995, non-clipped RBFT went from 158 g kg⁻¹ on 12 September, to 249 g kg⁻¹ by 24 October; non-clipped BFT went from 75 to 191 g kg⁻¹ over the same period. The difference between 1994 and 1995 was that clipping RBFT significantly lowered ($P < 0.05$) taproot TNC concentration on 29 August and 12 September. The differences between clipped and non-clipped plants only occurred on these two dates. By the final sampling of 1995, there were no differences ($P > 0.05$) in taproot TNC concentration between clipped and non-clipped RBFT.

The taproot TNC concentration at the final sampling

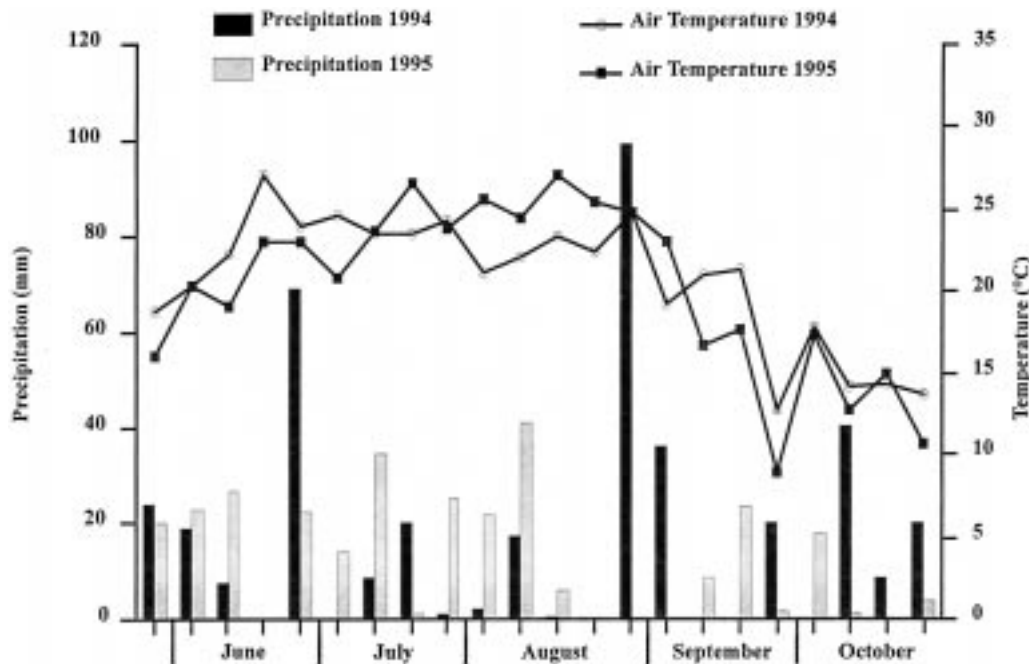


Fig. 3. Weekly average air temperature and precipitation from late May through October of 1994 and 1995 at the Agronomy Research Center near Columbia, MO.

was similar ($P > 0.05$) between 1994 and 1995 within individual treatments. Considering that shoot and root growth was limited by relatively dry weather in the autumn of 1995, one might have expected taproot TNC to be lower in 1995 than in 1994. Our data suggest that taproot TNC is higher priority sink for birdsfoot trefoil than is shoot or root growth, and thus taproot TNC is maintained at the expense of root or shoot growth under drought conditions. This is consistent with the findings of Hall et al. (1988), who showed that taproot TNC in alfalfa was maintained at the expense of shoot growth under drought conditions. The relative importance of taproot TNC for the persistence of perennial legumes perhaps contributes to its conservation during dry weather.

The taproot TNC concentrations and accumulation patterns we describe for BFT are similar to those reported by Nelson and Smith (1968) and Davis et al. (1995), with TNC concentration in early-summer between 50 and 100 g kg⁻¹ and late-autumn near 200 g kg⁻¹. The elevated levels of TNC in RBFT taproots combined with its lower shoot mass, suggests that RBFT partitions relatively more photosynthate to below-ground organs, either for storage or to support rhizome growth.

Rhizome Expression

In mid-September of both years, RBFT plants began to form new rhizomes. The percentage of plants with rhizomes increased with time: 60% in mid-September, 81% in late-September and nearly 100% by mid-October. This is a greater expression than Wedderburn and Gwynne (1981) found for first-year seedlings of *L. uliginosis* where only 36% of plants produced rhizomes. Rhizome production for RBFT appears to be induced

by shortening daylengths coincident with autumn growing conditions. In greenhouse grown RBFT, Nualsri et al., (1998) reported that rhizomes were most often formed on mature plants when the daylength was less than 13 h d⁻¹.

In our study, RBFT rhizomes originated around the perimeter and under the surface of the crown. In addition, they varied greatly in number and size. Rhizomes formed in mid- and late-September were typically 1 to 5 cm in length, 2 mm in diameter, had few buds or branches, and appeared chlorotic. As rhizomes continued to grow into October, rhizomes often became longer, fleshier, and sympodially branched. Rhizomes remained predominately underground with approximately 15% emerging to form short, leafy shoots. These rhizomes were similar in appearance and size to those described by Li and Beuselinck (1996).

Number, Combined Length, and Dry Mass of Rhizomes

The number, combined length, and dry mass of rhizomes were not affected by clipping treatment and differed only between sampling dates (Table 2). The number of rhizomes per plant steadily increased from mid-September through late-October of both years, with plants having three to six times more rhizomes in 1994 than in 1995 (Table 2). The combined length of rhizomes followed a similar pattern. The combined length of RBFT rhizomes averaged 57 and 16 cm plant⁻¹ in mid-September of 1994 and 1995, respectively, and grew to 187 and 56 cm plant⁻¹ by late-October. The significant difference between years for both the number of rhizomes per plant and combined length of rhizomes is likely due to the drier and warmer conditions in 1995.

Although the number of rhizomes per plant and the

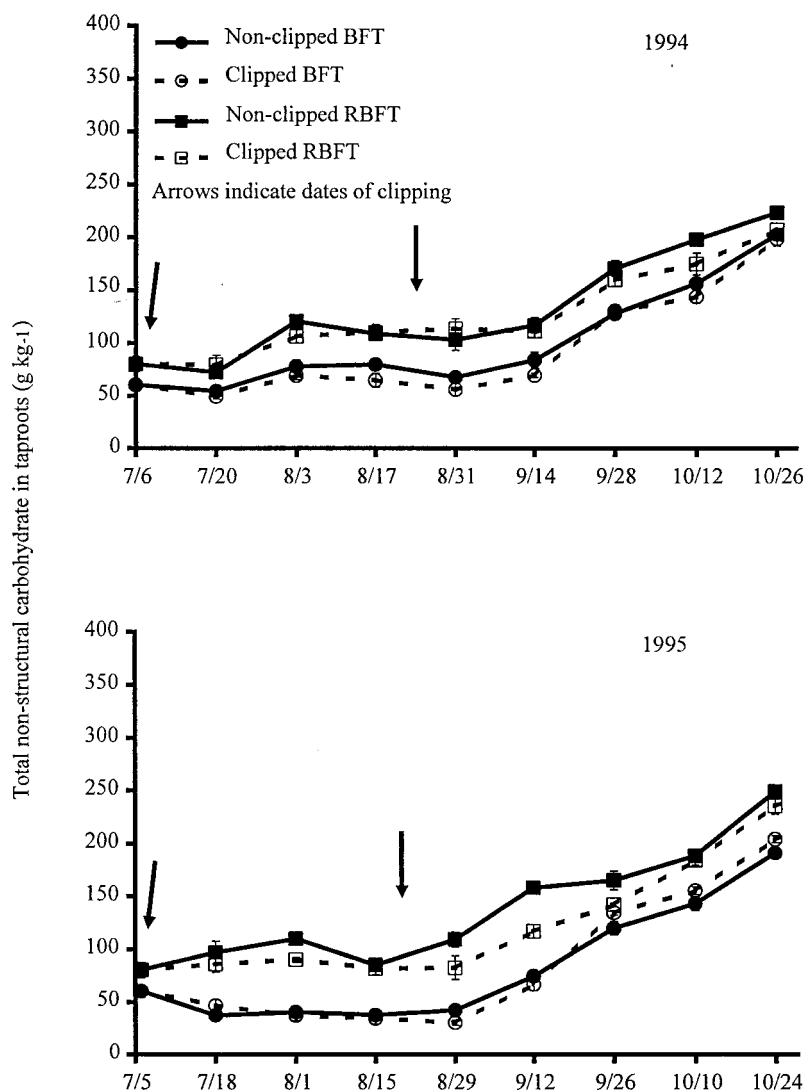


Fig. 4. Total nonstructural carbohydrate in taproots of non-clipped and clipped RBFT and BFT plants grown at the Agronomy Research Center, near Columbia, MO. Bars show standard errors. Arrows indicate dates of clipping.

combined length of rhizomes increased at each sampling after mid-September, rhizome dry mass did not increase significantly until October (Table 2). In 1994, rhizome dry mass was less than $0.23 \text{ g plant}^{-1}$ in mid- and late-September, but increased to $0.84 \text{ g plant}^{-1}$ by late-October. In 1995, rhizome dry mass followed a similar pattern, growing from 0.21 to $0.73 \text{ g plant}^{-1}$ over the same period. What is unique about rhizome dry mass is the lack of differences between 1994 and 1995. Although RBFT plants had fewer rhizomes per plant and less combined length in 1995, rhizome dry mass was unaffected. Apparently, RBFT adapted to the relatively dry and warm conditions of 1995 by making fewer, shorter but individually heavier rhizomes.

Rhizome TNC Concentration

The rhizome TNC concentration did not differ between years ($P > 0.05$), so data were combined across years. Clipping RBFT caused rhizome TNC to differ significantly only in mid-September (Table 3). At this

time, rhizomes from non-clipped and clipped plants had 171 and 119 g kg^{-1} TNC, respectively. The mid-September sampling was about 25 d after the last clipping. Apparently, clipped plants were not able to store as much TNC in their newly formed rhizomes, even though they did not differ from non-clipped plants in the other rhizome traits measured (Table 2). By late-September, rhizome TNC concentration of clipped RBFT plants was nearly identical to that for non-clipped plants. The fact that rhizome TNC concentration was equal between years, suggests that the heavier and shorter rhizomes in 1995 contained a greater mass of carbohydrate per rhizome than in 1994. This combined with the minimal response to clipping, suggests that the conservation of TNC in rhizomes maybe a survival mechanism for RBFT and is not surprising considering that the Moroccan parents of RBFT were collected in an arid region where herbivory is almost constant (Beuselinck, 1989).

There are reports of clipping not affecting the TNC concentration in the rhizomes of other species. Clipping *Trifolium ambiguum* M. Bieb three or five times per

Table 2. Rhizomes per plant, combined length of rhizomes, and rhizome dry mass from RBFT during 1994 and 1995. Data combine clipped and non-clipped treatments.

| Sampling date† | Year | | LSD (0.05) |
|------------------------------|-----------------------------|------|------------|
| | 1994 | 1995 | |
| | — no. plant ⁻¹ — | | |
| Rhizomes plant ⁻¹ | | | |
| Mid-September | 17 | 3 | 7 |
| Late-September | 25 | 5 | 6 |
| Mid-October | 33 | 10 | 8 |
| Late-October | 47 | 12 | 19 |
| LSD (0.05) | 13 | 3 | |
| | — cm plant ⁻¹ — | | |
| Combined length of rhizomes | | | |
| Mid-September | 57 | 16 | 18 |
| Late-September | 75 | 19 | 25 |
| Mid-October | 110 | 41 | 28 |
| Late-October | 187 | 56 | 85 |
| LSD (0.05) | 52 | 15 | |
| | — g plant ⁻¹ — | | |
| Rhizome dry mass | | | |
| Mid-September | 0.14 | 0.21 | NS |
| Late-September | 0.22 | 0.18 | NS |
| Mid-October | 0.45 | 0.48 | NS |
| Late-October | 0.84 | 0.73 | NS |
| LSD (0.05) | 0.28 | 0.21 | |

† In 1994, the sampling dates were 14 September, 28 September, 12 October and 26 October for mid-September, late-September, mid-October and late-October, respectively. In 1995, sampling dates were 12 September, 26 September, 10 October and 24 October for mid-September, late-September, mid-October and late-October, respectively.

year had little or no effect on the TNC concentration of crowns, taproots, or rhizomes (Peterson et al., 1994). They suggested the lack of response to clipping was due to the considerable belowground mass available for carbohydrate storage. Rhizomes of *T. ambiguum* comprise 45% of the total belowground mass (Peterson et al., 1994), where rhizomes of RBFT in this study made up <5% of total belowground mass.

SUMMARY AND CONCLUSIONS

The shoot mass of RBFT was about half that of BFT by the end of the growing season in both years. Root mass for all treatments was similar by late October of both years, although root mass was greater in 1994 than in 1995. The taproots from RBFT had greater amounts of TNC than BFT taproots throughout the growing season in both years. Compared with BFT, RBFT partitions photosynthates to root storage and rhizome production, rather than to shoot growth. The RBFT plants exhibited vigorous production of rhizomes during autumn and all plants had rhizomes present by mid-October. The ability of RBFT to increase rapidly rhizome and root growth, while at the same time increasing the TNC concentration in these same tissues, resulted in a rapid increase in total belowground TNC pools in the fall. Similar trends in other rhizomatous species were reported by Sheath (1981) for *L. uliginosis*, by Gabrielsen et al. (1985) for cicer milkvetch (*Astragalus cicer* L.), and by Saldivar et al. (1992) for rhizoma peanut (*Arachis glabrata* Benth).

Clipping RBFT plants twice during the summer did not affect rhizome growth. The failure of clipping to decrease rhizome production, combined with signifi-

Table 3. Total nonstructural carbohydrate (TNC) concentration of rhizomes from non-clipped and clipped RBFT. Data are combined over 1994 and 1995.

| Sampling date† | Non-clipped | Clipped | LSD (0.05) |
|----------------|--------------------|---------|------------|
| | g kg ⁻¹ | | |
| Mid-September | 171 | 119 | 27 |
| Late-September | 183 | 188 | NS |
| Mid-October | 206 | 208 | NS |
| Late-October | 221 | 220 | NS |
| LSD (0.05) | 30 | 29 | |

† In 1994, the sampling dates were 14 September, 28 September, 12 October and 26 October for mid-September, late-September, mid-October and late-October, respectively. In 1995, sampling dates were 12 September, 26 September, 10 October, and 24 October for mid-September, late-September, mid-October, and late-October, respectively.

cantly higher levels of below-ground TNC, may give RBFT the ability to withstand frequent defoliation better than other cultivars of birdsfoot trefoil. Further research is needed to quantify the contribution of rhizomes to stand persistence and forage yield after the establishment year.

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REFERENCES

- Beuselinck, P.R. 1989. *Lotus* spp. expedition in Morocco. *Lotus Newsl.* 20:31.
- Beuselinck, P.R. 1994. The rhizomes of *Lotus corniculatus* L. p. 215–219. In P.R. Beuselinck and C.A. Roberts (ed.) *Proc. The first Int. Lotus Symposium*, St. Louis, MO. March 22–24, 1994. Univ. Missouri Ext., Columbia, MO.
- Beuselinck, P.R., and W.F. Grant. 1995. Birdsfoot trefoil. p. 237–248. In R.F. Barnes et al. (ed.) *Forages Vol. 1, An Introduction to grassland agriculture*. Iowa State University Press, Ames, IA.
- Beuselinck, P.R., E.J. Peters, and R.L. McGraw. 1984. Cultivar and management effects on stand persistence of birdsfoot trefoil. *Agron. J.* 76:490–492.
- Davis, D.K., R.L. McGraw, P.R. Beuselinck, and C.A. Roberts. 1995. Total nonstructural carbohydrate accumulation in roots of annual lespedeza. *Agron. J.* 87:89–92.
- Gabrielsen, B.C., D.H. Smith, and C.E. Townsend. 1985. Cicer milkvetch and alfalfa as influenced by two cutting schedules. *Agron. J.* 77:416–422.
- Greub, L.J., and W.F. Wedin. 1971. Leaf area, dry matter production and carbohydrate reserve levels of birdsfoot trefoil as influenced by cutting height. *Crop Sci.* 11:734–738.
- Hall, M.H., C.C. Sheaffer, and G.H. Heichel. 1988. Partitioning and mobilization of photoassimilate in alfalfa subjected to water deficits. *Crop Sci.* 28:964–969.
- Li, B., and P.R. Beuselinck. 1996. Rhizomatous *Lotus corniculatus* L.: II. Morphology and anatomy of rhizomes. *Crop Sci.* 36:407–411.
- Nelson, C.J., and D. Smith. 1968. Growth of birdsfoot trefoil and alfalfa. III. Changes in carbohydrate reserves and growth analysis under field conditions. *Crop Sci.* 8:25–28.
- Nualrsi, C., P.R. Beuselinck, and J.J. Steiner. 1998. Rhizomatous *Lotus corniculatus* L.: III. Introgression of rhizomes into autogamous germplasm. *Crop Sci.* 38:503–509.
- Peterson, P.R., C.C. Sheaffer, R.M. Jordan, and C.J. Christians. 1994. Responses of kura clover to sheep grazing and clipping: II. Below-

ground morphology, persistence and total nonstructural carbohydrates. *Agron. J.* 86:660–667.

Peterson, P.R., C.C. Sheaffer, and M.H. Hall. 1992. Drought effects on perennial forage legume yield and quality. *Agron. J.* 84:774–779.

Saldívar, A.J., W.R. Ocumpaugh, R.R. Gildersleeve, and G.M. Prine. 1992. Total nonstructural carbohydrates and nitrogen of Florigrass rhizoma peanut. *Agron. J.* 84:439–444.

Sheath, G.W. 1981. *Lotus pendunculatus* - an agricultural plant? *Proc. N.Z. Grassl. Assoc.* 42:160–168.

Shenk, J.S., and M.O. Westerhaus. 1991. Population definition, sample selection and calibration procedures for near infrared reflectance spectroscopy. *Crop Sci.* 31:469–474.

Smith, D. 1962. Carbohydrate root reserves in alfalfa, red clover and birdsfoot trefoil under several management schedules. *Crop Sci.* 2:75–78.

Smith, D. 1981. Removing and analyzing total nonstructural carbohydrates from plant tissue. *Wisc. Agric. Exp. Stn. Publ.* 41.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. 2nd ed. McGraw-Hill, New York.

Wedderburn, M.E., and D.C. Gwynne. 1981. Production and nitrogen fixation in *Lotus uliginosus* under upland conditions in Southwest Scotland. *Ann. Bot. (London)* 48:5–13.

Effect of Drought on Growth, Carbohydrates, and Soil Water Use by Perennial Ryegrass, Tall Fescue, and White Clover

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ABSTRACT

In irrigated pastures of the semiarid, high-elevation western USA, perennial ryegrass (*Lolium perenne* L.) persistence is poor, and over time white clover (*Trifolium repens* L.) often dominates mixtures. Irrigation is often not available during autumn, when these perennial plants store carbohydrate reserves for spring regrowth. Our objective was to compare the effect of water stress on growth, carbohydrates, and soil water use of perennial ryegrass, white clover, and tall fescue (*Festuca arundinacea* Schreb.) in a greenhouse study. These three species were grown separately in a Kidman fine sandy loam, in 15-cm-diam, 1-m-deep pots and irrigated for 81 d (4 plants/pot). Paired pots were then either irrigated or subjected to water deficit (drought) for 30 d, followed by 10 d of recovery with irrigation. At 10-d intervals, four paired pots of each species were destructively sampled to determine leaf and storage organ dry matter and carbohydrate and simple sugar concentrations in storage organs. Root length density and soil water content were also sampled at 20-, 60-, and 90-cm soil depths. Leaf dry matter was lower in water-stressed plants than in irrigated plants by the end of the drought, but did not differ among species. After 10 d of recovery, storage carbohydrate concentration in droughted perennial ryegrass was lower than in white clover, and the ratio of simple sugars (droughted:irrigated) in perennial ryegrass was higher than in white clover. Tall fescue performed similarly to both species. Before the drought, grasses had similar, extensive root systems that withdrew more soil water from the 90-cm soil depth than did white clover. By the end of the 30-d drought, white clover had reduced soil water at all depths as much as the grasses. White clover survived drought and conserved carbohydrate reserves after 10 d of recovery better than did perennial ryegrass and similarly to tall fescue.

IN MANY TEMPERATE pasture environments, perennial ryegrass dominates mixtures with white clover; however, in rotationally stocked perennial ryegrass-white clover pastures of the semi-arid (43-cm average annual precipitation), high-elevation (1200 m), intermountain western USA, white clover often dominates perennial ryegrass. In an irrigated field study in northern Utah, where two varieties of perennial ryegrass were sown

with white clover and harvested mechanically to mimic a rotational grazing schedule, white clover dry matter averaged 55% of the total mixture yield over four harvest years (J. MacAdam et al., 2000, unpublished data). Poor persistence of perennial ryegrass is often attributed to lack of winter-hardiness, but may also be due to drought stress in autumn that is not apparent until spring growth is impaired. Compared with regions where perennial ryegrass is persistent, climatic conditions of the Intermountain West include moderate mean winter temperatures (–5 to –2°C), high summer daytime temperatures (25–30°C), low summer nighttime temperatures (8–11°C), low humidity, and autumn routine droughts when irrigation water is not available. This combination of conditions is rarely reported in the literature on intensively managed, cool-season grass pastures and, with the exception of the cool summer night temperatures, may contribute to the poor persistence of perennial ryegrass.

Tall Fescue

Tall fescue, another cool-season grass, appears to be more tolerant of the climate conditions typical of the Intermountain West (Jung et al., 1996; J. MacAdam et al., 2000, unpublished data), and was therefore included for comparison. In greenhouse and field drought studies under temperate conditions, tall fescue yielded more than perennial ryegrass and white clover, reportedly because leaf rolling prevented excessive stress (Johns, 1978; Johns and Lazenby, 1973). And compared with white clover, tall fescue's root system was more extensive below the 40-cm depth and extracted more soil water from 45- to 75-cm soil depth (Burch and Johns, 1978). Irrigated plots of tall fescue and white clover also yielded more than perennial ryegrass during the summer, in Armidale, New South Wales, Australia (at 1000 m, mean maximum daily temperature 28°C; Johns and Lazenby, 1973). Whether the *Acremonium*-fescue endophyte association contributed to tall fescue's drought tolerance is not known, as these studies were

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